PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Protocol of a prospective, multicenter phase I study to evaluate the safety, tolerability and preliminary efficacy of the bispecific PSMAxCD3 antibody CC-1 in patients with castration resistant prostate carcinoma
AUTHORS	Heitmann, Jonas S.; Walz, Juliane S.; Pflügler, Martin; Kauer, Joseph; Schlenk, Richard F.; Jung, Gundram; Salih, Helmut R.

VERSION 1 – REVIEW

REVIEWER	Urban Emmenegger Odette Cancer Centre Sunnybrook Health Sciences Centre University of Toronto 2075 Bayview Avenue Toronto, ON, M4N3M5 Canada
REVIEW RETURNED	14-Jun-2020

GENERAL COMMENTS	The manuscript by Heitmann et al is overall well written (aside from some typos), clearly structured, contains informative figures, and is considered suitable for publication in BMJ Open in that it is a 'study protocol for planned or ongoing research studies'. It describes an aggressive dose escalation protocol (including intra-patient dose escalation) of a novel PSMAxCD3 bispecific antibody (CC-1) with unique properties (NCT04104607). Given the expected acute nature of CC-1 related side effects, and accounting for the safety features incorporated in the protocol, the strategy chosen seems reasonably safe for participants. There are a few modifications the authors might consider: Page 8, line 108: as an interested reader one might wish to be given more information on how CC-1 is optimized compared to other bispecific antibodies. Introduction: it might be helpful to have a paragraph comparing bispecific antibody-based anti-PSMA strategies with other methods targeting PSMA (eg radioisotope therapy such as 177Lu-PSMA-617, antibody drug conjugates) Page 9, lines 137-140: the according statements should be supported by a reference. Inclusion criteria: 'CRPC after third line therapy' is a vague criteria; is there need for proof of biochemical and/or clinical or radiological disease progression at study enrolment? Figures 3 & 4: Figure 4 appears in the text before Figure 3, the reviewer suggests flipping the numbering of the Figures. Page 14/line 256: why is a PSA drop of 20% on day 15 of each cycle used as criteria to continue CC-1 treatment; both the timing (day 15) and degree of PSA drop (20%) are unusual (see PCWG3 criteria).

	Sample size expansion cohort: there is no justification for the number of pts of the expansion phase, ie 14 pts. Discussion (first paragraph): 'As of now, metastatic CRPC is an incurable and often rapidly progressive disease without effective treatment option' is too strong a statement; there are effective albeit non-curative treatment options for mCRPC in general; considering rephrasing. Because the manuscript may also be read by lay people/patients, there should be a prominent statement (eg in the Abstract) that the study treatment is taking place in the inpatient setting.

REVIEWER	Matthias Heck Technical University of Munich, Germany
REVIEW RETURNED	10-Jul-2020

GENERAL COMMENTS	This is an interesting study. I have the following questions: Is the trial listed in clinical trials? Or a similar database? Please report in the manuscript. Please clearly defined third-line therapy in the inclusion criteria: After Chemo and Abi or Enza? How is progression-free survival defined? PSA? Clinical? Radiographic? Please specify. How is PSMA-expression at baseline confirmed before treatment? 5-10% of PC patients don´t express PSMA in their tumor. Moreover, intraindividual heterogeneity with PSMA-positive and negative PC metastases has been reported in mCRPC. Second sentence of introduction: Please correct, currenty abiraterone, apalutamide or chemohormonal therapy are first-line standard treatments dependent on tumor volume in primary metastatic castration-sensitive prostate cancer.

VERSION 1 – AUTHOR RESPONSE

Reviewer #1:

The manuscript by Heitmann et al is overall well written (aside from some typos), clearly structured, contains informative figures, and is considered suitable for publication in BMJ Open in that it is a 'study protocol for planned or ongoing research studies'. It describes an aggressive dose escalation protocol (including intra-patient dose escalation) of a novel PSMAxCD3 bispecific antibody (CC-1) with
unique properties (NCT04104607). Given the expected acute nature of CC-1 related side effects, and accounting for the safety features incorporated in the protocol, the strategy chosen seems reasonably safe for participants.
There are a few modifications the authors might consider:

1. Page 8, line 108: as an interested reader one might wish to be given more information on how CC- is optimized compared to other bispecific antibodies.

Response:

Based on the suggestion of the reviewer, we provided additional information on CC-1 and its particular advantages in the “Methods and Analysis” section as follows:

Method and Analysis, page 7, lines 125-134
Properties of the bsAb CC-1
The bispecific PSMAxCD3 antibody CC-1 is an optimized IgG-like molecule (IgGsc format) with substantially improved serum half-life, especially when compared to the prototypical BiTE bsAb format. Specific modifications introduced in this proprietary format (disclosed patent application WO 2017/121905.) further reduce aggregation tendency and thus unspecific “off-target” T cell activation and immunogenicity. Its target is, in prostate carcinoma, expressed on both, tumor cells and tumor vessels. Vascular expression is expected to facilitate access of immune effector cells to the tumor site Notably, CC-1 binds a unique PSMA epitope which allows for such dual targeting not only in prostate carcinoma, but also in squamous cell carcinoma of the lung (data provided in the patent application).

Reference
Novel PSMA Binding Antibody And Uses Thereof. Patent application WO 2017/121905.

2. Introduction: it might be helpful to have a paragraph comparing bispecific antibody-based antiPSMA strategies with other methods targeting PSMA (eg radioisotope therapy such as 177Lu-PSMA617, antibody drug conjugates)

Response:

To address this comment of the reviewer, we have introduced a paragraph comparing CC-1 with other PSMA targeting therapies in the introduction as follows:

Introduction, page 5, lines 89-95

Novel strategies have to be developed to address the medical need of this patient population. Of particular interest in this context are strategies to target the prostate-specific membrane antigen (PSMA), which is expressed, at least to some extend, in almost all patients (up to 98%) with a highly tumor-restricted expression pattern. Targeted radiotherapy approaches using e.g. 177Lutetium-PSMA showed efficacy and a tolerable toxicity profile upon treatment of patients with metastatic disease. However, the duration of achieved responses is limited, and many patients do not at all benefit from this treatment option.

Introduction, page 6, lines 117-120
Of note, targeting PSMA with bsAbs not only holds promise to potentially induce more pronounced “immediate effects” compared to PSMA-targeted radiotherapy, but may also stimulate immunological memory and thus to mediate long term efficacy. In our first in human (FIH) study reported here, we evaluate CC-1 in patients with metastatic CRPC to determine overall safety and tolerability as well as the maximum tolerated dose (MTD) and first signs of efficacy.

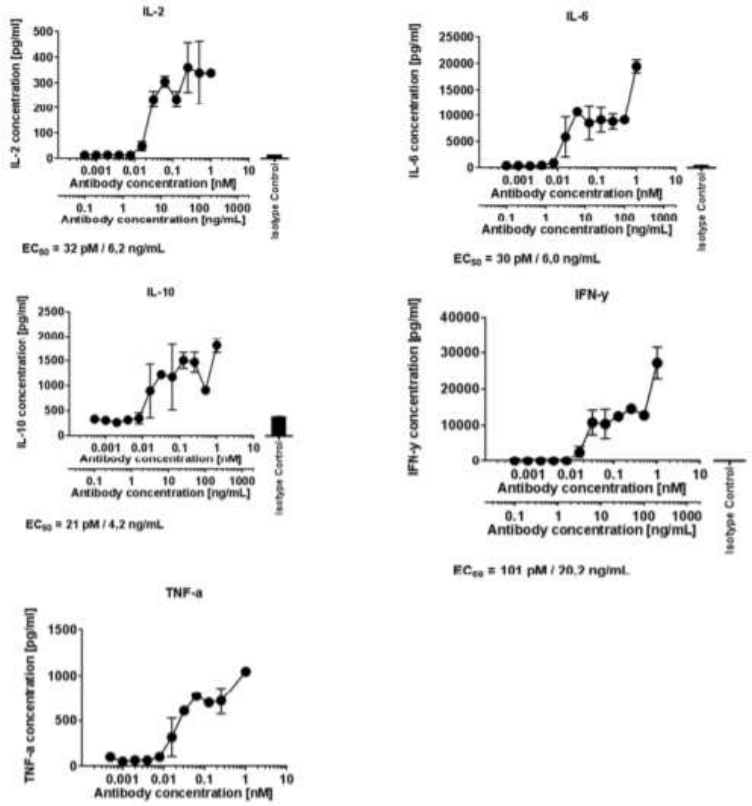
3. Page 9, lines 137-140: the according statements should be supported by a reference.

Response:

The statement on page Page 9, lines 137-140 relies on preclinical data that are presently under consideration for publication elsewhere (EMBO Mol. Med, in revision). The data are furthermore contained in the investigator brochure. We provide them below for information of the reviewer and have addressed this issue by amending the Methods and Analysis section as shown below.

Method and Analysis, page 8, lines 159-164
Definition of the starting dose (28 µg corresponding to 0.14 nMol) was based on preclinical data obtained with CC-1 and considerations taking into account available results of clinical studies with similar constructs: In vitro, CC-1 induced relevant T cell activation at concentrations of approximately 10 ng/ml. These data are provided in the investigators brochure of the study and contained in a manuscript reporting the preclinical characterization (Zekri et al, submitted).

Data for reviewer information:



Cytokine release induced by CC-1 in PBMC cultures co-incubated with PSMA-expressing tumor cells.
PBMC and LNCaP prostate carcinoma cells were incubated for 24 hours with the indicated concentrations of CC-1 and cytokines were detected in the supernatants of the cell mixtures using a Legendplex assay and flow cytometry. EC_{50} values were calculated by employing a non linear regression curve fit of a sigmoidal dose response curve. The table on the right summarizes the EC_{50} values generated with LNCaP cells (upper row) as well as those obtained with 22RV-1 cells (lower row) that express a lower number of PSMA molecules on the surface as determined by flow cytometry.

4. Inclusion criteria: 'CRPC after third line therapy' is a vague criteria; is there need for proof of biochemical and/or clinical or radiological disease progression at study enrolment?

Response:

We apologize by the reviewer for being vague with regard to this inclusion criteriion. For inclusion in our study, patients are required to have documented progressive disease prior to enrolment, as determined by rising tumor marker or radiological assessment. This is stated clearly in the study protocol, the manuscript has been amended to clarify this issue as follows

Method and Analysis, page 9, lines 199-201
∞ PaMents must be progressive prior to study enrolment, documented by either raising tumor marker or radiological assessment. Prior to study enrolment patients should have received chemotherapy and

abiraterone or enzalutamide.

5. Figures 3 & 4: Figure 4 appears in the text before Figure 3, the reviewer suggests flipping the numbering of the Figures.

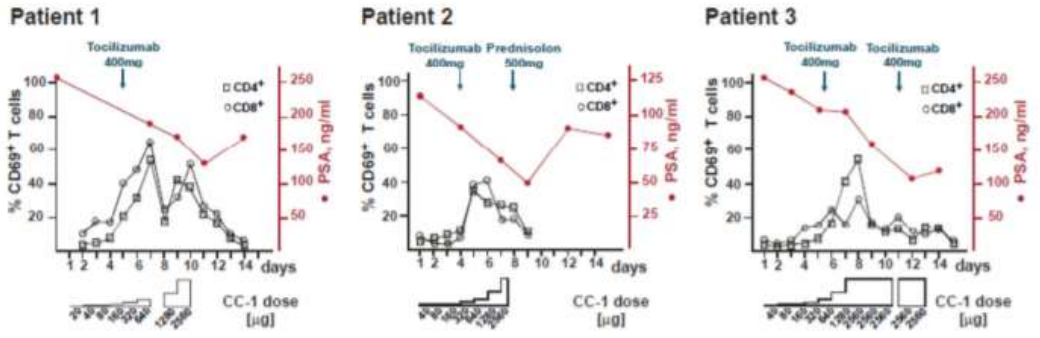
Response:

We thank the reviewer for raising this issue and apologize for providing the numbering not clear enough. We have inserted a reference to figure 3 right after figure 2 and thereby figure sequence matches the appearance in the manuscript.

Method and Analysis, pages 7-8, lines 148-152
This FIH study consists of two parts (Fig. 1): (i) a dose escalation part (patients n=10-72) comprising a dose escalation with prophylactic IL-6R blockade (8 mg/kg (body weight) tocilizumab) with intraindividual patient dose escalation (Fig. 2A-D) upon CC-1 induced dose limiting toxicity followed thereafter by a standard 3+3 dose escalation design without intra-individual dose escalation (Fig. 3).

6. Page 14/line 256: why is a PSA drop of 20% on day 15 of each cycle used as criteria to continue CC1 treatment; both the timing (day 15) and degree of PSA drop (20%) are unusual (see PCWG3 criteria).

We thank the reviewer for raising this issue and agree that this approach for definition of response may appear unusual. Indeed, according to PCWG3 criteria, it is recommended to disregard early rises of PSA (before 12 weeks) in determining PSA responses. However, we chose this criterion for the following reasons: Firstly, upon experimental individual treatment approaches conducted with CC-1 in prostate carcinoma patients prior to start of this trial, we observed PSA drops as early as 3 days after start of CC-1 infusion. Of note, these data are part of the upcoming publication mentioned above and are included for information of the reviewer below.



T cell activation and PSA levels upon treatment of prostate carcinoma patients with CC-1 monotherapy. CC-1 was applied in escalating doses as indicated. PSA values were monitored and T cell activation was assessed by flow cytometry.

In addition, our study concept requires inpatient treatment for at least 9 days for each cycle; in our view very rapid decision making whether patients may benefit from an additional cycle (again requiring hospitalization) is required to avoid undue burden for the patient. Considering the rapid response achieved in the so far treated patients, we think that PSA determination on day 15 can

serve as one (of several) factors to decision making.
Beside these reasons, bsAb in solid tumors have not been evaluated in far detail und it remains unclear, if time to response is longer or shorter than for hematologic diseases. However, as stated in the protocol, a patient might receive an additional treatment cycles, if he is considered to have experienced a benefit from CC-1 treatment. This is defined for example by the early response criterion (PSA drop on day 15 by 20% from baseline) and should help to guide investigator based decision. We slightly modified the section in the manuscript.

Method and Analysis, page 13, lines 282-286
If patients are considered to benefit from study treatment (indicated e.g. by a PSA drop of 20 % from baseline on day 15 to enable early decision making with regard to potential benefits in light of the required hospitalization to avoid undue burden for the patient), additional cycles of CC-1 may be applied to the same patient with the exact same dose schedule as in the previous cycle. Each patient may receive up to six cycles in total.

7. Sample size expansion cohort: there is no justification for the number of pts of the expansion phase, ie 14 pts.

Response:

We thank the reviewer for raising this important matter. The patient number is based on the objective to gain first signs of efficacy of CC-1 and to better define the recommended phase II dose. Based on the planning, in total 20 patients will be treated at the MTD level, six patients from the dose escalation phase and 14 patients of the expansion cohort. Thus we will be able to estimate within a single stage phase II design an objective response defined as PSA drop ≥50% estimating P0 the maximum response proportion of a poor drug of ≤ 30% of the patients and P1 the minimum response proportion of a good drug of ≥ 60% with a power of 80% and a type one error of 5%. To this aim we will need n=17 evaluable patients assuming a drop-out rate of 15% (n=3). The manuscript has been adapted accordingly.

Method and Analysis, page 15, lines 338-346
Subsequently, a dose expansion part with 14 patients is foreseen to gain more information about the MTD level and to better define the recommended phase II dose of CC-1. Based on the planning, in total 20 patients will be treated at the MTD level, six patients from the dose escalation phase and 14 patients of the expansion cohort. Thus we will be able to estimate within a single stage phase II design an objective response defined as PSA drop ≥50% estimating P0 the maximum response proportion of a poor drug of ≤ 30% of the patients and P1 the minimum response proportion of a good drug of ≥ 60% with a power of 80% and a type one error of 5%. To this aim we will need n=17 evaluable patients assuming a drop-out rate of 15% (n=3).

8. Discussion (first paragraph): ‘As of now, metastatic CRPC is an incurable and often rapidly progressive disease without effective treatment option’ is too strong a statement; there are effective albeit non-curative treatment options for mCRPC in general; considering rephrasing.

Response:

To address this comment of the reviewer, we amended the respective statement as follows

Discussion, page 18, lines 389-390
As of now, metastatic CRPC is an incurable and often rapidly progressive disease without curative treatment options.

9. Because the manuscript may also be read by lay people/patients, there should be a prominent statement (eg in the Abstract) that the study treatment is taking place in the inpatient setting.

Response:

We thank the reviewer for raising this important matter. To make this important information rapidly and clearly available, we amended the abstract as follows.

Abstract, page 2, lines 37-38
Each patient receives at least one cycle of CC-1 over a time course of 7 days in an inpatient setting.

Reviewer #2

This is an interesting study. I have the following questions:

1. Is the trial listed in clinical trials? Or a similar database? Please report in the manuscript.

Response:

We thank the reviewer for raising this issueand apologize for not stating clearly enough in the manuscript that this trial has been registered on clinicaltrials.gov and EudraCT. Besides the abstract, this is now additionally provided in the Methods and Analysis section of the revised manuscript as follows:

Abstract, pages 2-3, lines 51-53
Trial registration
This study is registered on clinicaltrials.gov (NCT04104607) and on clincialtrialsregister.eu (EudraCTNo.: 2019-000238-20).

Method and Analysis, page 16, lines 360-361
This study is registered on clinicaltrials.gov (NCT04104607) and on clincialtrialsregister.eu (EudraCTNo.: 2019-000238-20).

2. Please clearly defind third-line therapy in the inclusion criteria:
After Chemo and Abi or Enza?

Response:

We thank the reviewer for raising this important matter that also was raised by reviewer 1. Indeed, patients after chemotherapy and/or “second” generation androgen deprivation therapy can be included.
We included a short passage to describe the inclusion criterion in greater detail to address this issue as follows:

Method and Analysis, page 9, lines 199-201
∞ Patients must be progressive prior to study enrolment, documented by either rising tumor marker or radiological assessment. Prior to study enrolment patients should have received chemotherapy and

abiraterone or enzalutamide.

3. How is progression-free survival defined? PSA? Clinical? Radiographic? Please specify.

Response:

We apologize for not stating this information clearly in the study protocol. Progression free survival is defined as absence of radiologically confirmed progression of disease or death from any cause after first application of CC-1. This has been clarified in the manuscript.

Method and Analysis, page 14, lines 303-304
• Survival: Overall and progression free survival (absence of radiologically confirmed progression or death by any cause) evaluated by Kaplan-Meier method

4. How is PSMA-expression at baseline confirmed before treatment? 5-10% of PC patients don´t express PSMA in their tumor. Moreover, intraindividual heterogeneity with PSMA-positive and negative PC metastases has been reported in mCRPC.

Response:

We thank the reviewer for bringing up this issue. Indeed, in a substantial proportion of patients reduction of PSMA expression occurs over time. The most recent study addressing this issue we are aware of was conducted by Mannweiler and colleagues. They indeed observed PSMA negativity of metastatic lesions in 16% of patients, but only in one of the investigated 51 cases PSMA negativity in primary and metastatic lesions was observed. Notably, negativity was defined as PSMA expression between 0-10% on tumor cells (and thus not complete absence), and immunohistochemistry, which was employed in the analyses, has limited sensitivity to detect low expression levels, whereas as few as a few hundred molecules per cell suffice to render the malignant cells susceptible to T cell killing upon bispecific antibody (bsAb) treatment. In addition, targeting PSMA with bsAbs not only stimulates an immune response against the cells expressing the target molecule themselves, but also against potentially negative neighboring cells (so called bystander killing). These considerations led us initially not to require confirmation of PSMA expression in our phase I study. At present, we obtain information on PSMA expression in our patients “outside the study” by either results of a PSMA-PET conducted during standard of care and/or histopathological PSMA expression of a previously obtained tumor biopsy.

However, somewhat in line with the issue of the reviewer, we plan to obtain data from PSMA-PET for individuals who are recruited in the dose expansion phase of the study. A respective amendment will be send to competing authorities upon completion of the dose escalation phase.

To address the issue of the reviewer, the manuscript was amended as follows.

Introduction, page 5, lines 89-95
Of particular interest in this context are strategies to target the prostate-specific membrane antigen (PSMA), which is expressed, at least to some extend, in almost all patients (up to 98%) with a highly tumor-restricted expression pattern.

Discussion, page 19, lines 432-437
Of note, in the expansion phase we plan to implement PSMA-PET analysis to obtain reliable information on target antigen expression in patients undergoing analysis for efficacy, as reduction of PSMA expression on malignant cells reportedly occurs in a substantial proportion of patients over

time, even if few as a few hundred molecules per cell suffice to render the malignant cells susceptible to T cell killing upon bispecific antibody (bsAb) treatment.

References

Mannweiler S, Amersdorfer P, Trajanoski S, Terrett JA, King D, Mehes G. “Heterogeneity of Prostate Specific Membrane Antigen (PSMA) Expression in Prostate Carcinoma with Distant Metastasis.” Pathol. Oncol. Res. 15:167–172, 2009

5. Second sentence of introduction:
Please correct, currenty abiraterone, apalutamide or chemohormonal therapy are first-line standard treatments dependent on tumor volume in primary metastatic castration-sensitive prostate cancer.

Response:

We fully agree with the reviewer that this sentence should be changed and we inserted the information provided by the reviewer in the revised manuscript.

Introduction, page 5, lines 74-77
Currently, first-line therapy of metastatic castration-sensitive prostate cancer is conducted with androgen deprivation therapy (ADT) such as abiraterone or apalutamide or chemotherapy and depends on total tumor volume. However, frequently prostate carcinoma develops resistance to first line therapy.

VERSION 2 – REVIEW

REVIEWER	Urban Emmenegger Odette Cancer Centre Sunnybrook Health Sciences Centre 2075 Bayview Avenue Toronto, ON, M4N3M5 Canada
REVIEW RETURNED	18-Aug-2020

GENERAL COMMENTS	Introduction Lines 74 -77: In the reviewer’s mind the revised version (Currently, first-line therapy of metastatic castration-sensitive prostate cancer is conducted with androgen deprivation therapy (ADT) such as abiraterone or apalutamide or chemotherapy and depends on total tumor volume.) is less accurate than the original version (Androgen deprivation therapy (ADT) continues to be the first-line treatment for patients with advanced prostate cancer: (1) Strictly speaking ADT (ie chemical castration) is not the same as using abiraterone/ apalutamide, and chemotherapy definitely is not ADT; (2) enzalutamide is another treatment option for metastatic castration-sensitive prostate cancer (mCSPC); (3) while tumor volume is used in some constituencies as a criteria for whether to use docetaxel chemotherapy (ie in high-volume patients only), it does not inform on the use

	of abiraterone (used in men with high-risk mCSPC), or apalutamide/enzalutamide. I would keep it simple, eg: Androgen deprivation therapy (ADT) is the main component\ of standard of care first-line therapy of advanced prostate cancer. … Line 120: Should read … but may also stimulate immunological memory and thus mediate long term efficacy, instead of: … and thus to mediate long term efficacy. Methods and Materials Line 161: Definition of the starting dose (28 µg corresponding to 0.14 nMol) was based on preclinical data data obtained with CC-1 … Line 164: … contained in a manuscript reporting the preclinical characterization of CC-1. Discussion Lines 391-292: As of now, metastatic CRPC is an incurable and often rapidly progressive disease without curative treatment options.

REVIEWER	Matthias Heck Technical University of Munich, Germany
REVIEW RETURNED	10-Aug-2020

GENERAL COMMENTS	Please resubmit the paper with a reply letter answering all questions from reviewers and indicating the changes made in the manuscript. Thanks

VERSION 2 – AUTHOR RESPONSE

Reviewer #1:

1. Lines 74 -77: In the reviewer's mind the revised version (Currently, first-line therapy of metastatic castration-sensitive prostate cancer is conducted with androgen deprivation therapy (ADT) such as abiraterone or apalutamide or chemotherapy and depends on total tumor volume.) is less accurate than the original version (Androgen deprivation therapy (ADT) continues to be the first-line treatment for patients with advanced prostate cancer:

(1) Strictly speaking ADT (ie chemical castration) is not the same as using abiraterone/apalutamide, and chemotherapy definitely is not ADT;

(2) enzalutamide is another treatment option for metastatic castration-sensitive prostate cancer (mCSPC);

(3) while tumor volume is used in some constituencies as a criteria for whether to use docetaxel chemotherapy (ie in high-volume patients only), it does not inform on the use of abiraterone (used in men with high-risk mCSPC), or apalutamide/enzalutamide.

I would keep it simple, eg: Androgen deprivation therapy (ADT) is the main component\of standard of care first-line therapy of advanced prostate cancer

Response:

To address this comment, we have changed the wording as suggested by the reviewer to:

Introduction, page 5, lines 74-75

Androgen deprivation therapy (ADT) is standard of care first-line therapy of advanced prostate cancer.

2. Line 120: Should read … but may also stimulate immunological memory and thus mediate long term efficacy, instead of: … and thus to mediate long term efficacy.

Response:

To address this comment of the reviewer, we have changed the wording as suggested to:

Introduction, page 6, lines 117-119

Of note, targeting PSMA with bsAbs not only holds promise to potentially induce more pronounced "immediate effects" compared to PSMA-targeted radiotherapy, but may also stimulate immunological memory and thus mediate long term efficacy

3. Line 161:

Definition of the starting dose (28 µg corresponding to 0.14 nMol) was based on preclinical data data obtained with CC-1 …

Response:

To address this comment of the reviewer, we have omitted the double word:

Methods and Materials, page 8, lines 159-161

Definition of the starting dose (28 µg corresponding to 0.14 nMol) was based on preclinical data obtained with CC-1 and considerations taking into account available results of clinical studies with

similar constructs:

4. Line 164:

… contained in a manuscript reporting the preclinical characterization of CC-1.

Response:

We have addressed this comment of the reviewer as suggested:

Methods and Materials, page 8, lines 162-164

These data are provided in the investigators brochure of the study and contained in a manuscript reporting the preclinical characterization of CC-1 (Zekri et al, submitted).

5. Lines 391-292:

As of now, metastatic CRPC is an incurable and often rapidly progressive disease without curative treatment options.

Response:

To address this comment of the reviewer, we have amended the wording as proposed:

Discussion, page 18, lines 390-391

As of now, metastatic CRPC is an incurable rapidly progressive disease without curative treatment options.